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14. ABSTRACT

We demonstrate that transforming growth factor beta 1 (TGF-•1), a common growth factor in the bone marrow, reduces the adhesion of metastatic prostate cancer (PC) cells to bone marrow endothelial cells (BMEC), but increases the adhesion of these same cells to collagen type I, a major component of the bone matrix. Also, we show that metastatic PC cells are inhibited by TGF-•1 in a dose dependent fashion. The expression of TGF-•1 receptors type I and II are increased in metastatic PC cells and these observations provide a unique opportunity to use mathematical modeling to decipher the complex TGF-•1 signaling pathway required for PC metastasis. We are well on our way to understanding this pathway with the hope of using the information gained to better access the metastatic potential of PC.

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Introduction:

Approximately 28,000 men with prostate cancer (PC) will die from the metastatic disease (http://www.cancerresearch.org/prostatebook.html). Typically, the Gleason system is used as a histological grading system for PC and a predictor of cancer behavior; however, for patients with moderate Gleason scores, the clinical behavior is difficult to assess and more reliable prognostic indicators are needed to predict the metastatic potential of PC in these patients (1). One potential prognostic indicator is the production of growth factors and the response of PC cells to them. This study will focus on the complex role of transforming growth factor (TGF)-β1 in the metastatic behavior of PC cells. We will compare the effects of TGF- β1 on adhesion to bone-marrow endothelium, collagen, and growth, using non-bone-metastatic LNCaP cells and bone-metastatic C4-2 cells.

Body:

Our first objective described in specific aim 1A was to confirm that TGF-β1 reduces the adhesion of PC cells to human bone marrow endothelial cells. While doing this study, it was determined that our HBME cells were of canine origin and not human as originally thought. Thus, another source of human bone-marrow endothelial cells was acquired and the study was continued. The data demonstrated that TGF-β1 reduced the adhesion of C4-2 to BMEC but not LNCaP (**figure 1A**). Preliminary data testing the effect of adhesion of the highly metastatic PC cell line, PC-3, to BMEC under shear stress and TGF-β1 stimulation, revealed that TGF-β1 increases PC-3 adhesion to BMEC, the opposite of what is seen in our static adhesion assays **(figure 1B)**. These observations were only present when the PC cells were treated. Because it took about 9 months to get a new line of BMEC, this work is still ongoing and will be statistically analyzed for the final report and publication.

In specific aim 1B, we evaluated the effect of TGF-β1 on adhesion of PC cells with varying metastatic potential for bone to collagen type I, a major component of the bone matrix **(figure 2**). The ability of LNCaP cells to bind collagen type I was not altered by TGF-β1 treatment; however, adhesion of both C4-2B4 and PC-3 to collagen type I was increased by TGF-β1 treatment. C4-2B4 adhesion was increased by 14% and PC-3 cell adhesion to collagen type I was increased by 27%.

In specific aim 1C, we evaluated the effect of TGF-β1 on the growth of PC cells over a period of 7 days **(figure 3)**. The data generated showed that LNCaP growth was not altered by the doses of TGF-β1 used **(figure 3a)**; however, growth of the LNCaP-

derived C4-2 and C4-2B4 cells was reduced consistently at a TGF-β1 concentration of 10ng/ml **(figures 3b and c)**. C4-2B4 growth was reduced also at 0.1 and 1ng/ml of TGF-β1. This suggests that the PC cells derived from the bone microenvironment may be more sensitive to TGF-β1. To further test this, we treated PC-3 cells with TGF-β1 **(figure 3d)**. The growth of PC-3s was reduced only after 7 days of TGF-β1 treatment, but not to the degree of C4-2B4. The reduction in PC-3 cellular growth was approximately 25% compared to the control at all doses of TGF-β1 while the reduction in C4-2B4 cellular growth was 60% compared to the control at 10ng/ml of TGF-β1, and 35% compared to control at 0.1 and 1 ng/ml of TGF-β1. Because men with metastatic PC are treated with hormone ablation, we now realize that it is not relevant to consider the use of androgen in this study of PC bone metastasis. Instead, we decide to identify the mechanism for the difference in the non-bone metastatic PC cell response to TGF-β1 and bone metastatic PC cells. This information would also be more useful for the mathematical model currently being developed. Preliminary Western analysis showed that TGF-β1 receptor type I and II were preferentially expressed in PC cells derived from the bone-marrow (PC-3 and C4-2B4) compared to nonmetastatic LNCaP cells (data not presented).

The task for specific aim 2 was delayed because a new bone-marrow endothelial cell line had to be obtained and briefly characterized, and because the Co-PI had to replace the graduate student assigned to the project. Nevertheless, we have obtained some information based on the data presented above. The LNCaP Progression Model of increasingly metastatic lineage-related PC cells was used to study the effects of TGF-β1 on metastasis and to construct a dynamic mathematical model used to quantify TGF-β1-stimulated bone-metastatic potential of PC cells at different stages in metastasis **(Fig. 4)**. The experimental results show, among other things, that at the cell population level, TGF-β1 appears to regulate growth as well as adhesion to bone marrow endothelium differently in nonmetastatic and metastatic PC cells: (1) growth is inhibited in C4-2 and C4-2B4 cells but not in LNCaP; (2) there is a decrease in the TGF-β1-induced adhesion of C4-2 and C4-2B4 to BMEC; and, (3) there is no change in PC adhesion to TGF-β1-treated BMEC, suggesting that the changes in adhesion are the result of activation of TGF-β1 signaling in the PC cells, and not BMEC. In addition, Western blot data show that the classic TGF-β Type II receptor is expressed in all PC cells and present at higher levels in the bone-derived PC cells. Additional results show that Smad 2, one of the main effectors in the pathway, is activated in both LNCaP and C4-2, with earlier and more robust activation in LNCaP.

We have developed a mathematical model of TGF-β signaling in normal epithelial cells via Smad proteins and validated the model with literature data. The model, which consists of 14 non-linear ordinary differential equations (ODEs), 17 states and 39 parameters, is able to predict changes in the cytoplasmic and nuclear abundance of the Smad proteins in response to changes in the concentration of the TGF-β ligand. **Figure 5** shows an example comparison of the model prediction of total Smad2 abundance against literature data.

We have also analyzed the model extensively, using its predictions to investigate potential sources of abnormal signaling behavior that may be indicative of cancer progression. For example, the model indicates that a decrease in the number of receptors (perhaps via early genetic loss) in conjunction with an increase in the rate of Smad4 degradation is sufficient to drive tumor progression by reducing the TGF-β-induced growth-inhibition. These and other such findings are to be published in a manuscript under preparation.

Key Research Accomplishments:

The following are the key research accomplishments to date.

1. TGF-β1 reduces the adhesion of bone metastatic PC cells to BMEC
2. TGF- β1 increased the adhesion of bone metastatic PC cells to collagen type I
3. TGF- β1 preferentially reduced the proliferation of PC cells derived from the bone marrow
4. TGF- β1 receptor type I is preferentially expressed in PC cells derived from the bone marrow
5. TGF- β1 receptor type II is preferentially expressed in PC cells derived from the bone marrow in a linear progression model.
6. A detailed, validated, mathematical model that predicts cytoplasmic and nuclear abundance of the Smad proteins in response to changes in the amount of TGF-β ligand.
7. The model indicates, among other things, quantitatively how TGF-β-induced growth-inhibition is reduced as a result of a decrease in the level of receptors and an increase in the rate of Smad4 degradation.

Reportable Outcomes:

Grant Funded:

"*Transforming growth factor beta1 alters prostate cancer cell-bone marrow endothelium adhesion under shear stress*". Agency: The University of Delaware Research Foundation.

Abstracts presented at national meetings and details regarding a manuscript in preparation:

1. **Identifying the role of TGF-B in prostate cancer metastasis to bone.** Fayth Miles, Karla Boyd, Bianca Graves, Robert A. Sikes, **Babatunde Ogunnaike**, and **Carlton R. Cooper.** ABRCMS, 2005.
2. **The role of TGF-β1 in prostate cancer progression.** Miles FL, **Cooper CR**, **Ogunnaike B**, Sikes RA, Sequeira L, Graves B, Boyd K., 95th Annual Meeting of the American Association for Cancer Research (AACR), Washington D.C., April 1-5, 2006. Cancer Res 47: #3249

3. **Effects of TGF-β on Prostate Cancer Cell Adhesion to Bone Endothelium under Static and Flow Conditions.** Lewis, II C.M., Miles F.L., Molligan J., DeGraff D.J., Sikes R.A., **Cooper C.R**., HHMI, DoD Summer student research symposium; University of Delaware, August 9, 2006.
4. **TGF-Beta1 regulation of prostate cancer adhesion to bone marrow. Fayth Miles*,** Jeremy Molligan, **Babatunde Ogunnaike**, Robert Sikes, and **Carlton Cooper**. Poster 59, page 16 SBUR 2006 Annual Fall Meeting Program Book & Abstracts.
5. **TGF-β1 Regulation of Prostate Cancer (PCa) Adhesion to Bone. Fayth L. Miles,** Jeremy Molligan, **Babatunde Ogunnaike**, Ken van Golen, Robert A. Sikes, and **Carlton Cooper.** Poster, AACR 2007
6. **Modeling and Analysis of the TGF-β Signaling System**, Seung-Wook Chung, Fayth Miles, Mary C. Farach-Carson, **Carlton R. Cooper** and **Babatunde A. Ogunnaike**; (manuscript in preparation)

Conclusion:

We conclude at this point, that indeed, the effect TGF-β1 has on PC cell behavior varies and seems to depend on the cells' metastatic phenotype. This may be regulated in part by the differential expression of TGF-β1 receptors type I and II in PC cells, particularly the increased expression of these receptors in PC cells disseminated to the bone marrow. The reduction in bone metastatic PC cell adhesion to BMEC suggests that the timing and concentration of TGF-β1 released in the bone marrow stimulates the detachment of PC cells from BMEC to promote transendothelial migration (TEM) or tumor extravasation. Currently, we are in the process of exploring this theory. We are also able to quantify, in a dynamic mathematical model, the role of TGF- β1 signaling in tumor progression and the effect of various determinants, such as receptor level and the kinetics of various Smad protein activities on tumor progression. Lastly, we hope that at the end of this study, we are able to demonstrate that these receptors, along with TGF-β1-regulated adhesion molecules and matrix proteins may serve as targets to treat patients with PC metastasis to the skeleton.

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1) Ang, J., M. Lijovic, L.K. Ashman, K. Kan, and A.G. Frauman. **2004.** CD151 protein expressionpredicts the clinical outcome of low-grade primary prostate cancer better than histologic grading: a new prognostic indicator? Cancer Epidemiol Biomarkers Prev, **13**: p. 1717-21.
2) Teicher, B.A. **2001.** Malignant cells, directors of the malignant process: role of transforming growth factor-beta. Cancer Metastasis Rev, **20**: p. 133-43.
3) Bello-DeOcampo, D. and D.J. Tindall. **2003.** TGF-betal/Smad signaling in prostate cancer. Curr Drug Targets, **4**: p. 197-207.

Appendices

Figure 1A: The effect of TGF-β1 on PC cell adhesion to BMEC.

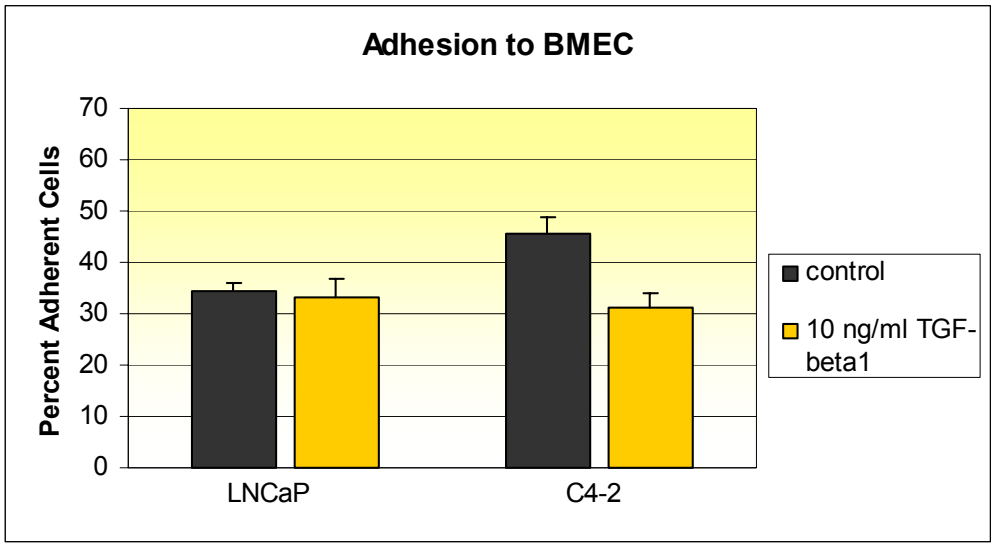


Figure 1B: TGF-β1 increased PC cell adhesion to BMEC under shear stress. Calcein labeled-PC-3 cells are show as white dots on a monolayer of BMEC under fluorescent microscope.

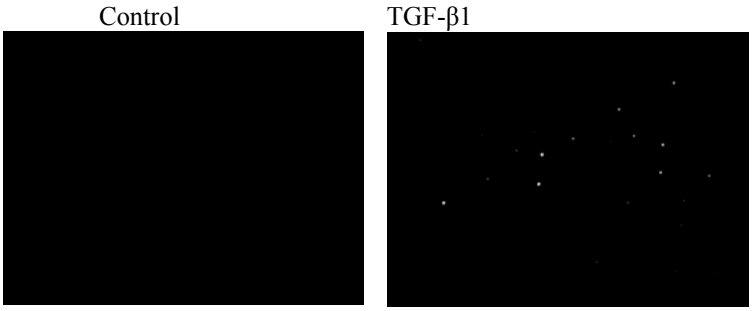


Figure 2: The effect of TGF-β1 on PC cells adhesion to collagen type I

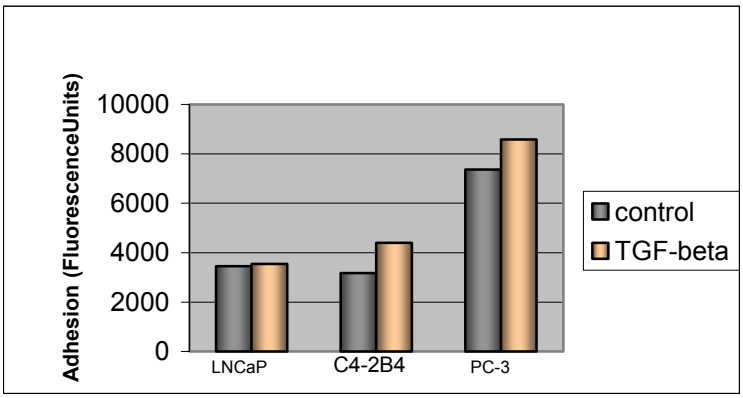
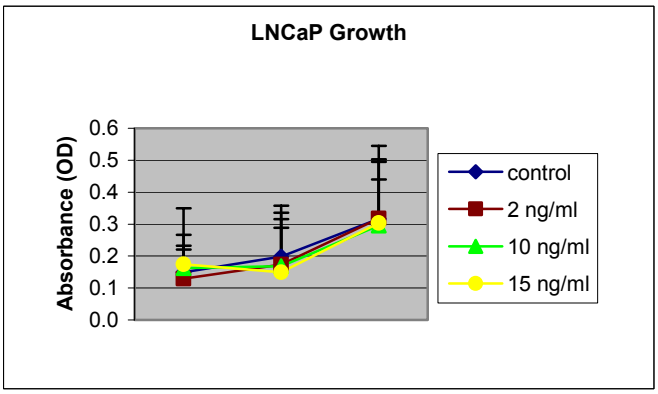
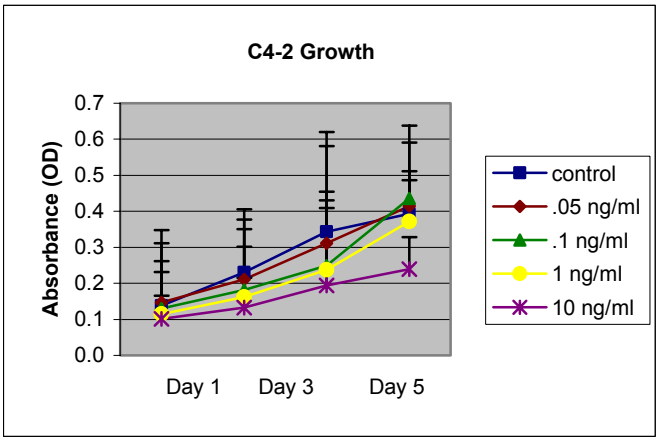


Figure 3: The effect of TGF-β1 on the growth of several PC cell lines with varying metastatic ability for bone

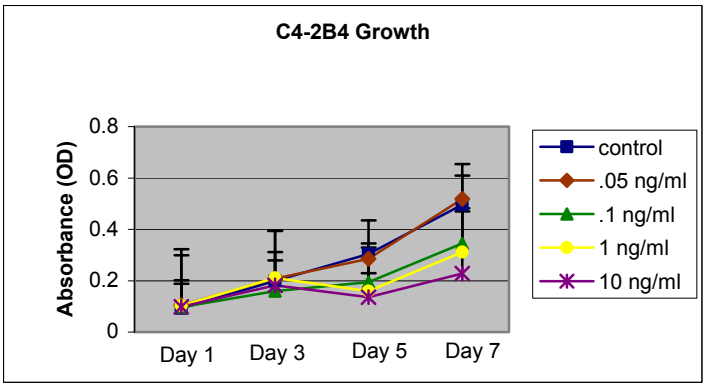
3A. Non-bone metastatic LNCaP cells



3B. Metastatic to mouse bone C4-2 cells



3C. Mouse bone-derived C4-2B4 cells



3D. Human bone-derived PC-3 cells

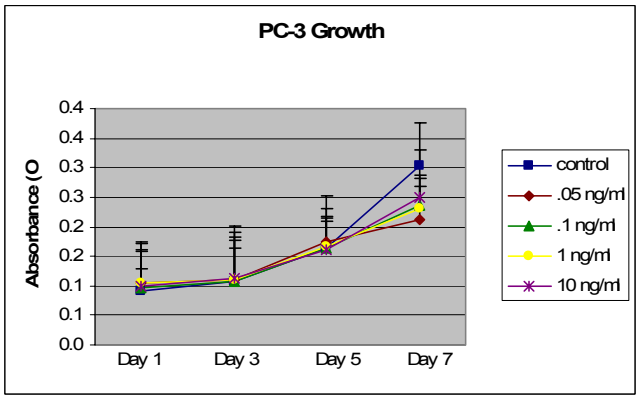


Figure 4: Discrete states in PC progression

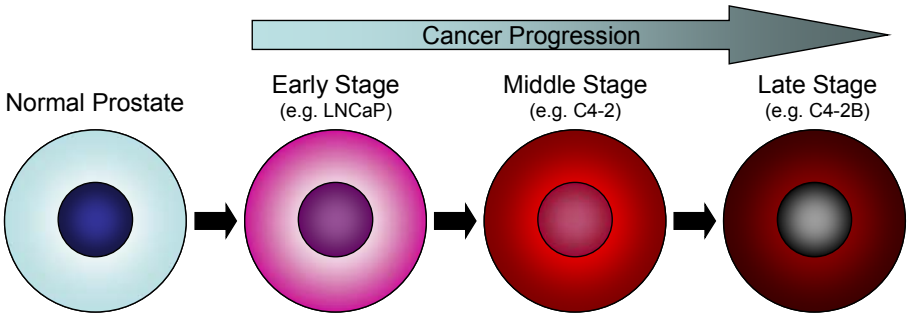


Figure 5: Mathematical model prediction versus literature data.